

The opinion in support of the decision being entered today was not written
for publication and is not binding precedent of the Board.

Paper No. 30

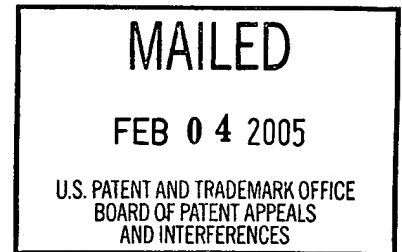
UNITED STATES PATENT AND TRADEMARK OFFICE

**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Ex parte JOHN H. LEE, JOSEPH D. HAHN and ROBERT C. MUSSER

Appeal No. 2003-2147
Application No. 09/392,243

ON BRIEF



Before SCHEINER, MILLS, and GREEN Administrative Patent Judges.

MILLS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. §134 from the examiner's final rejection of claims 21-27 and 36-54, which are all of the claims pending in this application.

Claims 21, 36 and 42 are illustrative of the claims on appeal and read as follows:

21. A method for preserving mucosa tissue comprising mixing a quantity of mucosa tissue with a preserving agent selected from the group consisting of hydrogen peroxide and phosphoric acid to yield the preserved mucosa tissue.

36. A method of treating mucosa tissue, the method comprising combining the mucosa tissue and a peroxide-containing compound to form an intermediate.

Appeal No. 2003-2147
Application No. 09/392,243

42. The method of claim 36, the method further comprising mixing the peroxide-containing compound and the mucosa tissue to form a mucosa product, the concentration of the peroxide-containing compound remaining in the mucosa product being undetectable when the concentration of the peroxide-containing compound in the mucosa product is determined using KMnO_4 titration.

The prior art references cited by the examiner are:

Van Gorp et al (Van Gorp)	5,607,840	Mar. 4, 1997
Balslev et al (Balslev)	4,438,100	Mar. 20, 1984
Oles	4,415,451	Mar. 20, 1979

Reference cited by the Merits Panel:

Hiles et al. (Hiles)	6,666,892	Dec. 23, 2003 (filed May 27, 1999)
----------------------	-----------	---------------------------------------

Grounds of Rejection

Claim 42 stands rejected under 35 U.S.C. §112, second paragraph for indefiniteness.

Claims 21-27 and 36-54 stand rejected under 35 U.S.C. §103(a), as obvious over Van Gorp in view of Balslev and Oles.

We reverse these rejections.

DISCUSSION

35 U.S.C. §112, second paragraph

Claim 42 stands rejected under 35 U.S.C. §112, second paragraph for indefiniteness.

Appeal No. 2003-2147
Application No. 09/392,243

As set forth in Amgen Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991):

The statute requires that “[t]he specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.” A decision as to whether a claim is invalid under this provision requires a determination whether those skilled in the art would understand what is claimed. See Shatterproof Glass Corp. v. Libbey-Owens Ford Co., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985) (Claims must “reasonably apprise those skilled in the art” as to their scope and be “as precise as the subject matter permits.”).

Furthermore, claim language must be analyzed “not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary skill in the pertinent art.” In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971).

The examiner argues that claim 42 is confusing because “the claim initially recites addition of a peroxide-containing compound to a composition, but then recites that the added product must become undetectable when the amount of added product is measured. It is therefore unclear how much peroxide-containing product can be added, if any.” Answer, page 3.

Appellants argue that claim 42 is not indefinite. Brief, page 38. Appellants argue that

[i]f the concentration of the peroxide containing compound remaining in the mucosa product, when determined using KMnO_4 titration, is not “undetectable” (i.e. is “detectable”) or if the particular subject matter does not entail “mixing the peroxide containing compound and the mucosa tissue to form a mucosa product” as claim 42 requires, then the person knows the particular subject matter falls outside the literal scope of claim

Appeal No. 2003-2147
Application No. 09/392,243

42. If, on the other hand, the concentration of the peroxide-containing compound remaining in the mucosa product, when determined using KMnO_4 titration, is "undetectable" and the particular subject matter does entail "mixing the peroxide-containing compound and the mucosa tissue to form a mucosa product," as claim 42 requires, then the person knows the particular subject matter falls within the literal scope of claim 42.

Brief, page 39.

In our view the examiner has failed to establish a prima facie case of indefiniteness. In our view, the examiner has failed to fully analyze the claim 42 language in view of the application disclosure.

For example, Example 1 of the specification states that first porcine mucosa was heated. A specific amount of hydrogen peroxide was added to the solution followed by mixing for 10 minutes. "The color of the solution changed so that it was similar to the color of a whey protein solution. The solution was analyzed to determine whether there was any hydrogen peroxide remaining. This was carried out by adding 0.01 M KMnO_4 to the diluted solution (1:150) and observing whether a color change took place. This test indicated that there was no hydrogen peroxide remaining in the product."

Specification, page 5.

Upon review of this portion of the specification, and the other examples 2-8 set forth in the specification, in our view, one of ordinary skill in the art would have been provided with an amount of hydrogen peroxide and an equivalent, to be added to the solution. This amount of hydrogen peroxide, according to the examples, provides for

Appeal No. 2003-2147
Application No. 09/392,243

an amount which, in some instances, is undetectable by KMnO_4 titration.¹

Furthermore, appellants concede that “if the concentration of the peroxide containing compound remaining in the mucosa product, when determined by KMnO_4 titration, is not “undetectable” (i.e. is “detectable”) ..., then the person knows the particular subject matter fall outside the literal scope of claim 42.” Brief, page 39.

In view of the above, when claim 42 is read in view of the specification the claim language is not indefinite. The rejection of claim 42 for indefiniteness is reversed.

35 U.S.C. §103(a)

Claims 21-27 and 36-54 stand rejected under 35 U.S.C. §103(a), as obvious over Van Gorp in view of Balslev and Oles.

In rejecting claims under 35 U.S.C. § 103, the examiner bears the initial burden of presenting a prima facie case of obviousness. See In re Rijckaert, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993). It is well-established that the conclusion that the claimed subject matter is prima facie obvious must be supported by evidence, as shown by some objective teaching in the prior art or by knowledge generally available to one of ordinary skill in the art that would have led that individual to combine the relevant teachings of the references to arrive at the claimed invention. See In re Fine, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988).

¹ The examiner also appears to understand that the prior art, Balslev, Column 6, lines 39-68, teaches that hydrogen peroxide continuously decomposes over time, eventually to a concentration of zero. Answer, page 11.

According to the examiner, Van Gorp teaches a process wherein mucosal protein hydrolysate is added to protein-containing food or feed. Van Gorp also discloses the preservation of the mucosa starting material using well known preservatives. Answer, page 4; Final Rejection, page 3. The examiner finds that Van Gorp differs from the claimed invention in that Van Gorp does not use the claimed peroxide or phosphoric acid as a preservative. Id.

To make up for this deficiency in Van Gorp, the examiner relies on Balslev and Oles for the teaching that both phosphoric acid and peroxide were well known preservatives in food and/or pharmaceutical applications. Thus, according to the examiner, the claimed substitution of well known preservatives for those used in Van Gorp must be considered an obvious substitution of one known equivalent preservative for another. Id., at 4. The examiner continues that, "the artisan of ordinary skill at the time of applicant's invention would have had a reasonable expectation from Oles and Balslev that phosphoric acid and/or peroxide would have functioned equivalently to the preservatives disclosed by Van Gorp, the artisan of ordinary skill would have been motivated to have substituted Oles' phosphoric acid and/or Balslev's peroxide for the preservatives disclosed by Van Gorp." Answer, pages 4-5.

Appellants respond, arguing, "there is no teaching or suggestion in the Van Gorp patent to use any preservative other than an oxygen scavenger, an antioxidant, or in low acidity environments, calcium propionate. ... In this regard, the Van Gorp patent principally mentions use of sodium metabisulfite as an oxygen scavenger, while also

Appeal No. 2003-2147
Application No. 09/392,243

referencing use of calcium propionate, BHT, and BHA as alternatively acceptable preservatives..." Brief, page 13. Appellants argue that "[t]he Van Gorp patent suggests the use of oxygen scavengers as preservatives. The Van Gorp patent does not suggest the incorporation of either an oxidizer (such as hydrogen peroxide) or an acidifier (such as phosphoric acid) in the process disclosed in the Van Gorp patent." Brief, page 14.

In addition, appellants provide evidence, Exhibit D, Jay, James M., Modern Food Microbiology, pages 259-296 (1986), which according to appellants, demonstrates there is no support for the position that the Oles phosphoric acid "would have functioned equivalently to the preservatives disclosed by Van Gorp..." Brief, page 14.

Appellants argue that Oles relies on a synergistic combination of acidifying agents (a combination of (1) acetic acid or other organic acid and (2) phosphoric acid), whereas the Van Gorp patent principally relies on an oxygen scavenging stabilizer ... [and] demonstrates the wide difference in operating function between the preservatives of the Van Gorp patent and the preservatives of the Oles patent. Brief, page 14.

Finally, appellants argue that Balslev "actually teaches away from using hydrogen peroxide as a preservative in the process disclosed in the Van Gorp patent. The Van Gorp patent principally teaches preservation by scavenging oxygen from the mucosa tissue with oxygen scavengers. ... According to the Balslev patent the hydrogen peroxide serves an oxidizing bactericide. ... An oxidizing agent provides oxygen to an active site via a chemical reaction. Providing oxygen to mucosa tissue is

Appeal No. 2003-2147
Application No. 09/392,243

exactly the opposite result of that desired by the preservatives principally employed in the Van Gorp patent, namely scavenging oxygen." Brief, page 16.

In response, the examiner finds with respect to Oles, that the claims are open ended and do not exclude the combination of acetic acid and phosphoric acid as a preservative. While the examiner is correct in his interpretation of the scope of claim 21, what is missing from the examiner's analysis is why one of ordinary skill in the art would have been motivated to select the specific phosphoric acid and acetic acid preservative in combination with mucosa tissue. Oles provides that the combination of acetic acid and phosphoric acid has specific application to low acid food products. See abstract. The examiner has not established that mucosa tissue would be considered an equivalent to a low acid food product.

In view of the above, we agree with appellants that the examiner has failed to establish a prima facie case of obviousness on the prior art evidence before us. "To establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant." In re Kotzab, 217 F.3d 1365, 1369-70, 55 USPQ2d 1313, 1316 (Fed. Cir. 2000). An adequate showing of motivation to combine requires "evidence that 'a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.'" Ecolochem, Inc. v. Southern Calif. Edison Co., 227 F.3d 1361, 1375, 56 USPQ2d 1065,

Appeal No. 2003-2147
Application No. 09/392,243

1075 (Fed. Cir. 2000).

In the present case, the examiner has failed to provide sufficient evidence that one of ordinary skill in the art would readily substitute one preservative for another, in view of evidence present in the record that preservatives of the prior art in fact function differently than the claimed preservative. Moreover, the evidence of record reasonably appears to reasonably show that specific preservatives are for required for specific applications and thus one of ordinary skill in the art would not readily substitute one preservative for another. We particularly note appellants' Exhibit D, Tables 11-1 and 11-3, provides evidence that preservatives have distinct applications. From the examiner's perspective Table 11-1 of Exhibit D provides that certain preservative have multiple applications (Answer, page 7), but th examiner does not acknowledge the reference's teaching that other preservatives do not have general application or provide evidence that the specific claimed preservatives are known in the art to have general application, or particular application to the preservation of mucosa tissue.

In view of the above, we reverse the rejection of the claims over Van Gorp in view of Balslev and Oles.

Other Issue

Upon return of the application to the examiner, it is recommended that the examiner carefully review the disclosure of Hiles, U.S. Patent No. 6,666,892 (attached) and determine its relevance under 35 U.S.C. § 102(e) or 103(c), if any, to the claimed

Appeal No. 2003-2147
Application No. 09/392,243

subject matter. In particular, Hiles would appear to have a filing date, May 27, 1999, prior to the filing date of the present application, September 9, 1999. Hiles discloses the treatment of tissue mucosa with a disinfecting agent which is desirably an oxidizing agent, and preferably hydrogen peroxide. Col. 4, lines 44-67. Hiles states that the process will eliminate contaminants but will also produce a tissue which "exhibits no substantial degradation of physical and mechanical properties," i.e., the tissue will be preserved. (Col. 7, lines 1-8).

CONCLUSION

The rejection of claim 42 under 35 U.S.C. §112, second paragraph for indefiniteness is reversed. The rejection of claims 21-27 and 36-54 under 35 U.S.C. §103(a), as obvious in view of Van Gorp in view of Balslev and Oles is reversed.

The application is returned to the examiner to determine the relevance of Hiles to the claimed subject matter.

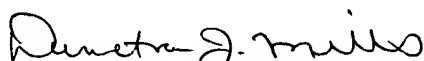
Appeal No. 2003-2147
Application No. 09/392,243

No time period for taking any subsequent action in connection with this appeal
may be extended under 37 CFR § 1.136(a).

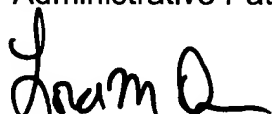
REVERSED



TONI R. SCHEINER
Administrative Patent Judge



DEMETRA J. MILLS
Administrative Patent Judge



LORA M. GREEN
Administrative Patent Judge

)
)
)
)
)
) BOARD OF PATENT
)
) APPEALS AND
)
) INTERFERENCES
)
)
)

Appeal No. 2003-2147
Application No. 09/392,243

WESTMAN CHAMPLIN & KELLY, P.A.
Suite 1800 International Centre
900 Second Avenue South
Minneapolis, MN 55402-3319



US00666892B2

(12) **United States Patent**
Hiles et al.

(10) **Patent No.:** US 6,666,892 B2
(45) **Date of Patent:** *Dec. 23, 2003

(54) **MULTI-FORMED COLLAGENOUS BIOMATERIAL MEDICAL DEVICE**

(75) Inventors: Michael C. Hiles, Lafayette, IN (US);
Umesh H. Patel, West Lafayette, IN (US)

(73) Assignee: Cook Biotech Incorporated, West Lafayette, IN (US)

(*) Notice: This patent issued on a continued prosecution application filed under 37 CFR 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/320,933

(22) Filed: May 27, 1999

(65) **Prior Publication Data**

US 2002/0099448 A1 Jul. 25, 2002

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/916,490, filed on Aug. 22, 1997, now Pat. No. 6,206,931.
(60) Provisional application No. 60/024,542, filed on Aug. 23, 1996, provisional application No. 60/024,693, filed on Sep. 6, 1996, and provisional application No. 60/110,407, filed on Dec. 1, 1998.

(51) Int. Cl.⁷ A61F 2/02

(52) U.S. Cl. 623/23.72; 623/925; 523/113

(58) Field of Search 623/11.11, 23.71,
623/23.61, 23.75, 924, 925, 1.34, 23.72;
523/105, 113

(56) **References Cited**

U.S. PATENT DOCUMENTS

2,127,903 A 8/1938 Bowen

3,346,401 A 10/1967 Barat et al.
3,649,163 A 3/1972 McCusker
4,292,972 A 10/1981 Pawelchak et al.
4,347,234 A 8/1982 Wahlig et al.
4,404,970 A 9/1983 Sawyer
4,412,947 A 11/1983 Cioca
4,606,337 A 8/1986 Zimmermann et al.
4,681,588 A 7/1987 Ketharanathan
4,755,593 A 7/1988 Lauren
4,798,611 A 1/1989 Freeman, Jr.
4,801,299 A 1/1989 Brendel et al.
4,813,958 A 3/1989 Dixon
4,837,379 A 6/1989 Weinberg
4,838,888 A 6/1989 Nashef

(List continued on next page.)

FOREIGN PATENT DOCUMENTS

WO	9522611	8/1995
WO	9624661	8/1996
WO	9625179	8/1996
WO	9631157	10/1996
WO	9632146	10/1996
WO	9822158	5/1998
WO	9825636	6/1998
WO	9825637	6/1998
WO	9826291	6/1998

OTHER PUBLICATIONS

Hodde, J.P. et al.: "Gycosaminoglycan Content of Small Intestinal Submucosa: A Bioscaffold for Tissue Replacement" Tissue Engineering, vol. 2, No. 3, 1996, pp. 209-217, XP002063373.

(List continued on next page.)

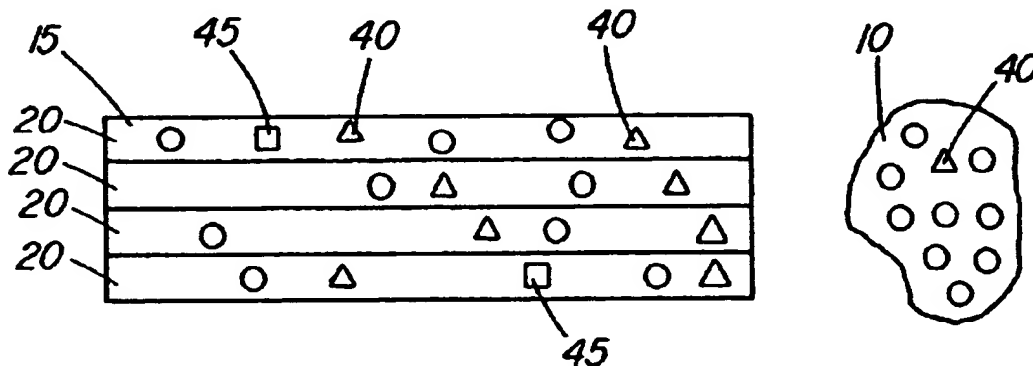
Primary Examiner—Paul B. Prebilic

(74) Attorney, Agent, or Firm—Woodard, Emhardt, Moriarty, McNett & Henry LLP

(57) **ABSTRACT**

The invention involves a submucosa tissue that has the capability of being shape formed or shape configured. The submucosa involves a purified form of submucosa tissue. Optionally, the submucosa can be packaged in such a manner to permit sterility or maintain sterility of the submucosa.

8 Claims, 1 Drawing Sheet



U.S. PATENT DOCUMENTS

4,885,005 A	12/1989	Nashef et al.	5,498,420 A	3/1996	Edgar et al.
4,891,359 A	1/1990	Saferstein et al.	5,501,706 A	3/1996	Arenberg
4,902,508 A	2/1990	Badylak et al.	5,507,810 A	4/1996	Prewett et al.
4,948,540 A	8/1990	Nigam	5,510,121 A	4/1996	Rhee et al.
4,950,483 A	8/1990	Ksander et al.	5,510,418 A	4/1996	Rhee et al.
4,956,178 A	9/1990	Badylak et al.	5,512,291 A	4/1996	Li
4,969,912 A	11/1990	Kelman et al.	5,514,181 A	5/1996	Light et al.
4,970,298 A	11/1990	Silver et al.	5,516,395 A	5/1996	Anhauser et al.
4,976,733 A	12/1990	Girardot	5,520,925 A	5/1996	Maser
4,994,084 A	2/1991	Brennan	5,522,840 A	6/1996	Krajicek
5,024,841 A	6/1991	Chu et al.	5,531,786 A	7/1996	Perry et al.
5,028,695 A	7/1991	Eckmayer et al.	5,531,791 A	7/1996	Wolfbarger, Jr.
5,094,661 A	3/1992	Levy et al.	5,554,389 A	9/1996	Badylak et al.
5,106,949 A	4/1992	Kemp et al.	5,565,210 A	10/1996	Rosenthal et al.
5,171,574 A	12/1992	Kuberasampath et al.	5,567,806 A	10/1996	Abdul-Malak et al.
5,206,023 A	4/1993	Hunziker	5,571,181 A	11/1996	Li
5,206,028 A	4/1993	Li	5,607,476 A	3/1997	Prewett et al.
5,215,541 A	6/1993	Nashef et al.	5,607,590 A	3/1997	Shimizu
5,219,576 A	6/1993	Chu et al.	5,645,860 A *	7/1997	Knapp, Jr. et al. 424/551
5,256,418 A	10/1993	Kemp et al.	5,711,969 A	1/1998	Patel et al.
5,275,826 A	1/1994	Badylak et al.	5,733,337 A	3/1998	Carr, Jr. et al.
5,279,612 A	1/1994	Eberhardt	5,755,791 A	5/1998	Whitson et al.
5,336,616 A	8/1994	Livesey et al.	5,762,966 A	6/1998	Knapp, Jr. et al.
5,352,463 A	10/1994	Badylak et al.	5,782,914 A *	7/1998	Schankereli 623/11
5,368,608 A	11/1994	Levy et al.	5,866,414 A	2/1999	Badylak et al.
5,372,821 A	12/1994	Badylak et al.	5,885,619 A	3/1999	Patel et al.
5,374,515 A	12/1994	Parenteau et al.	6,358,284 B1 *	3/2002	Fearnot et al. 623/23.72
5,397,352 A	3/1995	Burres			
5,411,887 A	5/1995	Sjolander			
5,413,791 A	5/1995	Rhee et al.			
5,437,287 A	8/1995	Phillips et al.			
5,445,833 A	8/1995	Badylak et al.			
5,447,536 A	9/1995	Girardot et al.			
5,451,406 A	9/1995	Lawin et al.			
5,456,693 A	10/1995	Conston et al.			
5,457,093 A	10/1995	Cini et al.			
5,460,962 A	10/1995	Kemp			
5,466,462 A	11/1995	Rosenthal et al.			
5,476,516 A	12/1995	Seifter et al.			
5,480,427 A	1/1996	Kelman et al.			
5,489,022 A	2/1996	Baker			
5,489,261 A	2/1996	Franzblau et al.			

OTHER PUBLICATIONS

Badylak, S.F., et al.; "The Use of Xenogeneic Small Intestinal Submucosa as a Biomaterial for Achille's Tendon Repair in a Dog Model"; Journal of Biomedical Materials Research, vol. 29, No. 8, 1995, pp. 977-985, XP002071882.

Cobb, M.A. et al.; "Histology After Dural Grafting With Small Intestinal Submucosa", Surgical Neurology, vol. 46, No. 4, 1996, pp. 389-394, XP002071883.

Vaught, J.D. et al.; "Detrusor Regeneration in the Rat Using Porcine Small Intestinal Submucosal Grafts: Functional Innervation and Receptor Expression", The Journal of Urology, vol. 155, No. 1, 1996, pp. 374-378, XP002071884.

* cited by examiner

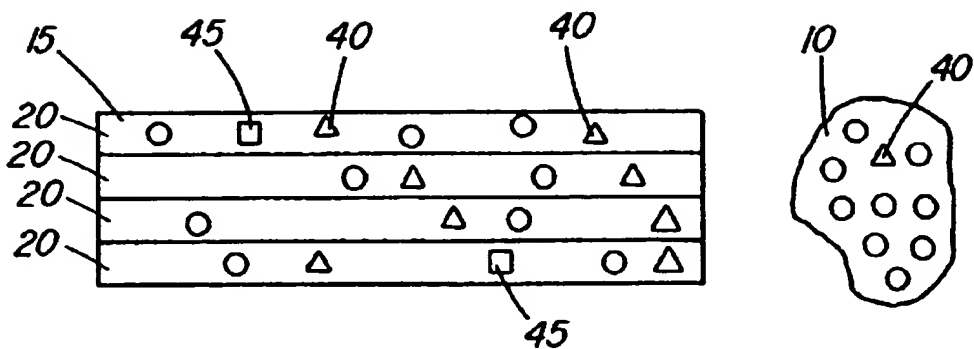


FIG. 1

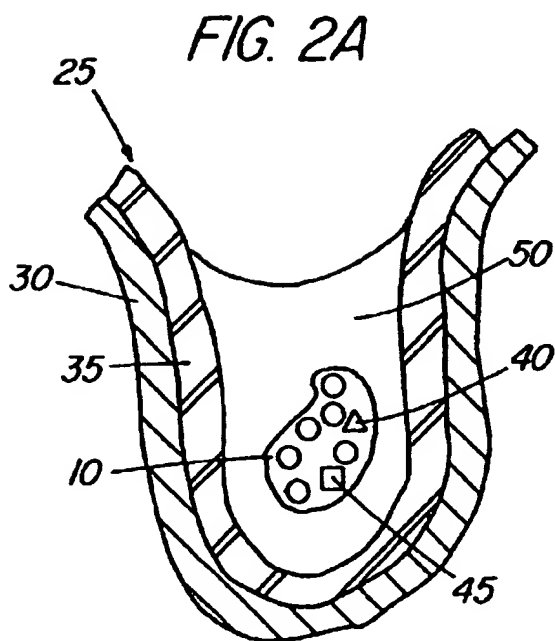


FIG. 2A

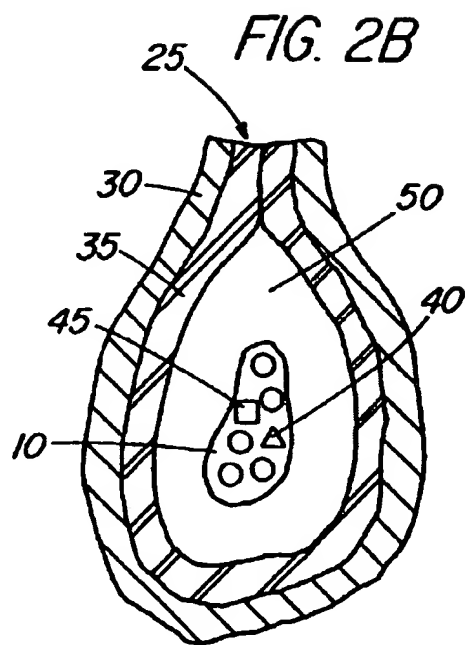


FIG. 2B

1

MULTI-FORMED COLLAGENOUS BIOMATERIAL MEDICAL DEVICE

STATEMENT OF RELATED APPLICATIONS

This application is a continuation in part of U.S. application Ser. No. 08/916,490, filed Aug. 22, 1997, now issued as U.S. Pat. No. 6,206,931, which claims the benefit of U.S. Provisional Application Serial No. 60/024,542 filed Aug. 23, 1996 and of U.S. Provisional Application Serial No. 60/024,693 filed, Sep. 6, 1996; this application also claims the benefit of U.S. Provisional Application Serial No. 60/110,407 filed Dec. 1, 1998.

TECHNICAL FIELD

The invention generally relates to a medical device and in particular, to a medical device comprising a collagenous biomaterial.

BACKGROUND OF THE INVENTION

A naturally occurring biomaterial for medical implantation is more desirable than a synthetic implant. Synthetic implants tend to cause adverse reactions in a patient, including thrombosis, immune responses, and potentially restenosis in vascular applications. Therefore, a medical implant that reduces or eliminates these problems is a technical advance.

Collagenous biomaterials are known to be used in medical applications as medical devices. As a naturally occurring biomaterial, the implant produces less complications than a synthetic implant. Collagen is used as an abundant source of protein, and is most notably derived from bovine skin. Collagen forms a matrix that is useable as an implant. However, as a biomaterial, it does not have good manipulation properties, unless treated in other ways. In addition, one problem with these material is that collagen biomaterials also carry with them antigens which cause an immune response in the patient. Therefore, a product that behaves like collagen in vivo yet is highly manipulative and elicits less to no negative immune response is a technical achievement.

SUMMARY OF THE INVENTION

The foregoing problems are solved and a technical advance is achieved with the present invention. A new biomaterial comprising the submucosa of a tissue was discovered to have greater benefit than using collagen. For example, the submucosa is shown to exhibit more remodeling, regrowth, and regeneration of tissue upon implant. It has been shown that submucosal tissue is absorbed by the patient and thus the patient does not require post-implantation procedures to remove the implant. The submucosal tissue has been shown to elicit favorable immune response that leads to an accommodation of the submucosal implant versus a rejection based response. Therefore, to further improve the submucosal tissue's industrial utility, the applicants have discovered that this utility can be achieved by improving the submucosa's purity and forming the submucosal tissue into various forms. Such forms, include, but are not limited to sheets, sponges, fluidized, etc. The present invention relates to a purified form of submucosal tissue that is treated in such a manner as to confer some shape memory and shape configuration to the implant. However, the submucosal tissues are not limited to implants and can be formed to be used in topical applications as well, such as wound dressings or wound plugs.

2

In addition, the problem of maintaining the sterility of the medical device is solved by including the medical device in a pouch or plurality of pouches. The pouches can be at least one of a gas permeable, sealed, hermetically sealed, sterile UV protected, and multiple pouched. In addition, the foregoing problems are solved by including processes of making the medical device.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 shows two aspects of the invention as the biomaterial multilaminate sheet or in sponge forms.

FIG. 2A shows one aspect of the invention as the biomaterial in a double pouched package, wherein the package is in an open configuration.

FIG. 2B shows one aspect of the invention as the biomaterial in a closed double pouched package.

DETAILED DESCRIPTION

In the discussions herein, a number of terms are used. In order to provide a clear and consistent understanding of the specification and claims, the following definitions are provided.

Bioburden—refers to the number of living microorganisms, reported in colony-forming units (CFU), found on and/or in a given amount of material. Illustrative microorganisms include bacteria, fungi, and their spores.

Disinfection—refers to a reduction in the bioburden of a material.

Sterile—refers to a condition wherein a material has a bioburden such that the probability of having one living microorganism (CFU) on and/or in a given section of the material is one in one-million or less.

Pyrogen—refers to a substance which produces febrile response after introduction into a host.

Endotoxin—refers to a particular pyrogen which is part of the cell wall of gram-negative bacteria. Endotoxins are continually shed from the bacteria and contaminate materials.

Purification—refers to the treatment of a material to remove one or more contaminants which occur with the material, for instance contaminants with which the material occurs in nature, and/or microorganisms or components thereof occurring on the material. Illustratively, the contaminants may be those known to cause toxicity, infectivity, pyrogenicity, irritation potential, reactivity, hemolytic activity, carcinogenicity and/or immunogenicity.

Biocompatibility—refers to the ability of a material to pass the biocompatibility tests set forth in International Standards Organization (ISO) Standard No. 10993 and/or the U.S. Pharmacopeia (USP) 23 and/or the U.S. Food and Drug Administration (FDA) blue book memorandum No. G95-1, entitled "Use of International Standard ISO-10993, Biological Evaluation of Medical Devices Part-1: Evaluation and Testing." Typically, these tests assay as to a material's toxicity, infectivity, pyrogenicity, irritation potential, reactivity, hemolytic activity, carcinogenicity, and/or immunogenicity. A biocompatible structure or material when introduced into a majority of patients will not cause an adverse reaction or response. In addition, it is contemplated that biocompatibility can be effected by other contaminants such as prions, surfactants, oligonucleotides, and other biocompatibility effecting agents or contaminants.

Contaminant—refers to an unwanted substance on, attached to, or within a material. This includes, but is not

3

limited to: bioburden, endotoxins, processing agents such as antimicrobial agents, blood, blood components, viruses, DNA, RNA, spores, fragments of unwanted tissue layers, cellular debris, and mucosa.

Tela submucosa—refers to a layer of collagen-containing connective tissue occurring under the mucosa in most parts of the alimentary, respiratory, urinary, integumentary, and genital tracts of animals.

The invention is generally directed to a medical device, comprising a collagenous biomaterial (also referred to as collagen-based matrices, tissue mucosa, tissue submucosa, intestines, biomaterial) and is further described in the non-limiting disclosure set forth below.

One such collagenous biomaterial includes tissue mucosa, which also further includes a tissue submucosa, which further includes a small intestine submucosa (SIS), also described herein as tela submucosa. Tela submucosa is a multi-laminate structure, comprising the tunica submucosa, lamina muscularis mucosa, and the stratum compactum. Collagenous materials can also have biotrophic agents comprising at least one of a proteoglycan, glycosaminoglycan, and growth factor. The SIS can be made using the techniques described in Cook et al., WIPO Publication WO 98/22158, dated May 28, 1998, which is the published application of PCT/US97/14855, the disclosure of which is set forth below.

One type of collagenous mucosa is tela submucosa, and as with many animal tissues, is generally aseptic in its natural state, provided the human or animal does not have an infection or disease. This is particularly the case since the tela submucosa is an internal layer within the alimentary, integumentary, respiratory, urinary, and genital tracts of animals. Accordingly, it is generally not exposed to bacteria and other cellular debris such as the epithelium of the intestinal tract. One feature of the present invention is the discovery that by disinfecting the source tissue for the tela submucosa prior to delamination, the aseptic state of the tela submucosa layer can be preserved or substantially preserved, particularly if the delamination process occurs under sterile conditions.

Other sources of mucosa exist. For example, the mucosa can also be derived from vertebrate liver tissue as described in WIPO Publication, WO 98/25637, based on PCT application PCT/US97/22727; from gastric mucosa as described in WIPO Publication, WO 98/26291, based on PCT application PCT/US97/22729; from stomach mucosa as described in WIPO Publication, WO 98/25636, based on PCT application PCT/US97/23010; or from urinary bladder mucosa as described in U.S. Pat. No. 5,554,389; the disclosures of all are expressly incorporated herein.

In particular, it has been discovered that disinfecting the tela submucosa source, followed by removal of a purified biomaterial including the tela submucosa, e.g. by delaminating the tela submucosa from the tunica muscularis and the tunica mucosa, minimizes the exposure of the tela submucosa to bacteria and other contaminants. In turn, this enables minimizing exposure of the isolated tela submucosa biomaterial to disinfectants or sterilants if desired, thus substantially preserving the inherent biochemistry of the tela submucosa and many of the tela submucosa's beneficial effects.

A tela submucosa implantable collagen biomaterial according to the present invention can, as indicated above, be obtained from the alimentary, respiratory, urinary, integumentary, or genital tracts of animals. Preferably, the tela submucosa tissues, which are collagen-based and thus predominantly collagen, are derived from the alimentary

4

tract of mammals, such as cows, sheep, dogs, and most preferably from the intestinal tract of pigs. A most preferred source of whole small intestine is harvested from mature adult pigs weighing greater than about 450 pounds. Intestines harvested from healthy, non-diseased animals will contain blood vessels and blood supply within the intestinal tract, as well as various microbes such as *E. coli* contained within the lumen of the intestines. Therefore, disinfecting the whole intestine prior to delamination of the tela submucosa substantially removes these contaminants and provides a preferred implantable tela submucosa tissue which is substantially free of blood and blood components as well as any other microbial organisms, pyrogens or other pathogens that may be present. In effect, this procedure is believed to substantially preserve the inherent aseptic state of the tela submucosa, although it should be understood that it is not intended that the present invention be limited by any theory. All that is required or be met is that the biomaterial satisfies the above-mentioned criteria.

It is also desirable that the collagenous biomaterial according to the present invention be substantially free of any antiviral agents or any antimicrobial type agents which can affect the biochemistry of the biomaterial and its efficacy upon implantation. In the past, one method of treating such tissue material is to rinse the delaminated tissue in saline and soak it in an antimicrobial agent, for example, as disclosed in U.S. Pat. No. 4,956,178. While such techniques can optionally be practiced with isolated collagenous mucosa or submucosa of the present invention, preferred processes according to the present invention avoid the use of antimicrobial agents and the like which can not only affect the biochemistry of the collagenous biomaterial but also can be unnecessarily introduced into the tissues of the patient.

As discussed above, it has been discovered that a highly pure form of an implantable tela submucosa collagen biomaterial can be obtained by first disinfecting a tela submucosa source prior to removing a purified collagen biomaterial including the tela submucosa layer, e.g. by delaminating the tela submucosa source. It has also been discovered that certain processing advantages as well as improved properties of the resultant tela submucosa layer are obtained by this process, including greater ease in removing attached tissues from the submucosa layer, and a characteristic, low contaminant profile.

Processes of the invention desirably involve first rinsing the tela submucosa source one or more times with a solvent, suitably water. The rinsing step is followed by treatment with a disinfecting agent. The disinfecting agent is desirably an oxidizing agent. Preferred disinfecting agents are peroxy compounds, preferably organic peroxy compounds, and more preferably peracids. Such disinfecting agents are desirably used in a liquid medium, preferably a solution, having a pH of about 1.5 to about 10, more preferably a pH of about 2 to about 6, and most preferably a pH of about 2 to about 4. In methods of the present invention, the disinfecting agent will generally be used under conditions and for a period of time which provide the recovery of characteristic, purified submucosa matrices as described herein, preferably exhibiting a bioburden of essentially zero and/or essential freedom from pyrogens. In this regard, desirable processes of the invention involve immersing the tissue source (e.g. by submersing or showering) in a liquid medium containing the disinfecting agent for a period of at least about 5 minutes, typically in the range of about 5 minutes to about 40 hours, and more typically in the range of about 0.5 hours to about 5 hours.

A preferred peroxy disinfecting agent is hydrogen peroxide. The concentration of hydrogen peroxide can range from

about 0.05% to 30% by volume. More preferably the hydrogen peroxide concentration is from about 1% to 10% by volume and most preferably from about 2% to 5% by volume. The solution can or can not be buffered to a pH from about 5 to 9. More preferably the pH is from about 6 to 7.5. These concentrations can be diluted in water or in an aqueous solution of about 2% to about 30% by volume alcohol. Most preferably the alcohol is ethanol. The solution temperature can range from about 15 to 50° C. More preferably the solution temperature is from about 20 to 40° C. Most preferably, the solution temperature is from about 32 to 37° C. The exposure time can range from about 10 to 400 minutes. Preferably, the exposure time is from about 120 to 240 minutes. More preferably, the exposure time is from 180 to 210 minutes.

A preferred organic peroxide disinfecting agent is perpropionic acid. The concentration of perpropionic acid can range from about 0.1% to 10% by volume. More preferably the perpropionic acid concentration is from about 0.1% to 1.0% by volume and most preferably from about 0.2% to 0.5% by volume. These concentrations of perpropionic acid can be diluted in water or in an aqueous solution of about 2% to about 30% by volume alcohol. Most preferably the alcohol is ethanol. The tela submucosa tissue source can be exposed to the organic peroxide solution for periods from about 15 minutes to about 40 hours, and more typically in the range of about 0.5 hours to about 8 hours. Other peroxy disinfecting agents are suitable for use as described in "Peroxygen Compounds", S. Block, in *Disinfection, Sterilization and Preservation*, S. Block, Editor, 4th Edition, Philadelphia, Lea & Febiger, pp. 167-181, 1991; and "Disinfection with peroxygens", M. G. C. Baldry and J. A. L. Fraser, in *Industrial Biocides*, K. Payne, Editor, New York, John Wiley and Sons, pp. 91-116, 1988.

Another oxidizing disinfecting agent is chlorhexidine (1,6-di(4-chlorophenyl)diguanido)hexane in its digluconate form. The concentration of chlorhexidine digluconate can range from about 0.1% to 15% by weight. More preferably, the chlorhexidine digluconate concentration is from about 0.1% to 2% by weight and most preferably from about 0.2% to 5% by weight. The solution can or can not be buffered to a pH from about 5 to 8. More preferably the pH is from about 5.5 to 7. These concentrations can be diluted in water or in an aqueous solution of about 2% to about 20% by volume alcohol. Most preferably the alcohol is ethanol at a concentration of about 5% to 10%. The solution temperature can range from about 15 to 30° C. The exposure time can range from about 10 to 400 minutes. More preferably the exposure time is from about 30 to 60 minutes. Other chlorine agents are described in "Chlorhexidine", G. W. Denton, in *Disinfection, Sterilization and Preservation*, S. Block, Editor, 4th Edition, Philadelphia, Lea & Febiger, pp. 274-289, 1991.

In preferred preparative processes, a peracid or other disinfecting agent can be dissolved in a dilute aqueous alcohol solution, preferably wherein the alcohol has from 1 to about 6 carbon atoms, and wherein the alcohol can generally comprise from about 1% to about 30% by volume of the solution. More preferred alcohols for use in the invention are selected from the group consisting of ethanol, propanols and butanols. Ethanol is a preferred alcohol for these purposes.

When a peracid is used in the disinfection, it is preferably selected from the group consisting of peracetic acid, perpropionic acid or perbenzoic acid. Peracetic acid is the most preferred disinfecting agent. The peracetic acid is preferably diluted into about a 2% to about 10% by volume alcohol

solution. The concentration of the peracetic acid can range, for example, from about 0.05% by volume to about 1.0% by volume. Most preferably the concentration of the peracetic acid is from about 0.1% to about 0.3% by volume. Hydrogen peroxide can also be used as a disinfecting agent. Alternatively, or in addition, the tela submucosa tissue source, e.g. from small intestine, can be disinfected utilizing disinfecting agents such as glutaraldehyde, formalin and the like, which are also known for their ability to introduce substantial crosslinking into collagen matrices, in contrast to the action of other disinfecting agents such as peracids which can be used to disinfect without introducing such crosslinking. Additionally, the tela submucosa source can be treated with radiation, e.g., gamma radiation, for purposes of disinfection.

Variations on the disinfection process can also include the following:

1. Intestine is treated with 0.2% peracetic acid, 5% ethanol solution at a ratio of 10:1 solution to intestine ratio by weight. Solution has a pH of 2.6. Solution and intestine are vigorously mixed for two hours.
2. Intestine is treated with 1% peracetic acid, 25% ethanol solution at a ratio of 5:1 solution to intestine ratio by weight. Solution has a pH of 2. Solution and intestine are vigorously mixed for one hour.
3. Intestine is treated with 1% peracetic acid, 15% ethanol, and 10% hydrogen peroxide solution at a ratio of 5:1 solution to intestine ratio by weight. Solution and intestine are vigorously mixed for one hour.
4. Whole small intestine is rinsed four times with high purity water for 15 minutes. The intestine is then subjected to 1.5 MRAD Electron Beam radiation.
5. Whole small intestine is rinsed four times with high purity water for 15 minutes. Lengthwise along a conveyor belt, the intestine is subjected to high-intensity pulsed light which disinfects the intestine.

Following the treatment as described above, the tela submucosa layer is delaminated from its source, e.g., whole intestine, cow uterus and the like. It has been found that by following this post-disinfection-stripping procedure, it is easier to separate the tela submucosa layer from the attached tissues, e.g. at least from attached tunica muscularis tissue, as compared to stripping the tela submucosa layer prior to disinfection. Moreover it has been discovered that the resultant tela submucosa layer in its most preferred form exhibits superior histology, in that there is less attached tissue and debris on the surface compared to a tela submucosa layer obtained by first delaminating the tela submucosa layer from its source and then disinfecting the layer. Moreover, a more uniform tela submucosa tissue can be obtained from this process, and a tela submucosa having the same or similar physical and biochemical properties can be obtained more consistently from each separate processing run. Importantly, a highly purified, substantially sterile tela submucosa is obtained by this process. The stripping of the tela submucosa source is preferably carried out by utilizing a disinfected or sterile casing machine, to produce a tela submucosa which is substantially sterile and which has been minimally processed. A suitable casing machine is the Model 3-U-400 Stridhs Universal Machine for Hog Casing, commercially available from the AB Stridhs Maskiner, Göteborg, Sweden. Therefore, the measured bioburden levels are minimal or substantially zero. Of course, other means for delaminating the tela submucosa source can be employed without departing from the present invention, including for example those means well known in the art, including delaminating by hand.

It has also been discovered that more preferred processes according to the present invention, not only will eliminate or significantly reduce contaminants contained in the tela submucosa collagen biomaterial, but also will produce a tissue which exhibits no substantial degradation of physical and mechanical properties, e.g., differential porosity (i.e. wherein one side of the submucosa layer has greater porosity than the other side), and good strength, for example burst strength. Also, it has been discovered that more preferred processes do not affect the differential porosity of the tela submucosa collagen biomaterial, which ultimately affects the level of efficacy of this tissue implant. For example, the tissue is not necessarily treated with a crosslinking agent or a material that disrupts the porosity or inherent, native structure of the collagen biomaterial. Moreover, when hydrogen peroxide is employed, the biomaterial as a whole has greater porosity as well as a higher oxygen content. This helps to ensure the absence of contaminants e.g., endotoxins, pyrogens, and the like.

Preferred collagen-based matrices of the invention, preferably submucosa-containing matrices, are also characterized by the low contaminant levels set forth in Table 1 below, each contaminant level taken individually or in any combination with some or all of the other disclosed contaminant levels. The abbreviations in Table 1 are as follows: CFU/g=colony forming units per gram; PFU/g=plaque forming units per gram; μ g/mg=micrograms per milligram; ppm/kg=parts per million per kilogram; and EU/g=endotoxin units per gram.

TABLE 1

FEATURE	FIRST PREFERRED LEVEL	SECOND PREFERRED LEVEL	THIRD PREFERRED LEVEL
ENDOTOXIN	<12 EU/g	<10 EU/g	<5 EU/g
BIOBURDEN	<2 CFU/g	<1 CFU/g	<0.5 CFU/g
FUNGUS	<2 CFU/g	<1 CFU/g	<0.5 CFU/g
NUCLEIC ACID	<10 μ g/mg	<5 μ g/mg	<2 μ g/mg
VIRUS	<500 PFU/g	<50 PFU/g	<5 PFU/g
PROCESSING AGENT	<100,000 ppm/kg	<1,000 ppm/kg	<100 ppm/kg

Even more preferred collagen-based matrices of the invention contain an endotoxin level of less than 1 EU/g, and most preferably less than 0.5 EU/g.

Purified collagen-based matrices according to the present invention can be processed in a number of ways, to provide collagenous matrices useful both in vitro and in vivo. For example, the submucosa can be configured to provide tissue grafts useful in vascular applications, e.g., as generally described in U.S. Pat. No. 2,127,903 and 4,902,508.

The tela submucosa of the invention possesses mechanical properties highly desirable for tissue graft materials in vascular applications, including low porosity index, high compliance, and a high burst strength. One skilled in the art will appreciate that the preferred tissue graft material will be of low enough porosity to prevent intraoperative hemorrhage and yet of high enough porosity to allow extension of a newly-developed vasa vasorum through the graft material to nourish the neointimal and luminal surface.

Tela submucosa tissue of the present invention can also be processed to provide fluidized compositions, for instance using techniques as described in U.S. Pat. No. 5,275,826. In this regard, solutions or suspensions of the tela submucosa can be prepared by comminuting and/or digesting the tela submucosa with a protease (e.g. trypsin or pepsin), for a period of time sufficient to solubilize the tissue and form

substantially homogeneous solution. The submucosa starting material is desirably comminuted by tearing, cutting, grinding, shearing or the like. Grinding the submucosa in a frozen or freeze-dried state is advantageous, although good results can be obtained as well by subjecting a suspension of pieces of the submucosa to treatment in a high speed blender and dewatering, if necessary, by centrifuging and decanting excess waste. The comminuted tela submucosa can be dried, for example freeze dried, to form a powder. Thereafter, if desired, the powder can be hydrated, that is, combined with water or buffered saline and optionally other pharmaceutically acceptable excipients, to form a fluid tissue graft composition, e.g. having a viscosity of about 2 to about 300,000 cps at 25EC. The higher viscosity graft compositions can have a gel or paste consistency.

Fluidized tela submucosa of this invention finds use as an injectable heterograft for tissues, for example, bone or soft tissues, in need of repair or augmentation most typically to correct trauma or disease-induced tissue defects. The present fluidized submucosa compositions are also used advantageously as a filler for implant constructs comprising, for example, one or more sheets of tela submucosa formed into sealed (sutured) pouches for use in cosmetic or trauma-treating surgical procedures.

In one illustrative preparation, tela submucosa prepared as described herein is reduced to small pieces (e.g. by cutting) which are charged to a flat bottom stainless steel container. Liquid nitrogen is introduced into the container to freeze the specimens, which are then comminuted while in the frozen state to form a coarse tela submucosa powder. Such processing can be carried out, for example, with a manual arbor press with a cylindrical brass ingot placed on top of the frozen specimens. The ingot serves as an interface between the specimens and the arbor of the press. Liquid nitrogen can be added periodically to the tela submucosa specimens to keep them frozen.

Other methods for comminuting tela submucosa specimens can be utilized to produce a tela submucosa powder usable in accordance with the present invention. For example, tela submucosa specimens can be freeze-dried and then ground using a manual arbor press or other grinding means. Alternatively, tela submucosa can be processed in a high shear blender to produce, upon dewatering and drying, a tela submucosa powder.

Further grinding of the tela submucosa powder using a prechilled mortar and pestle can be used to produce a consistent, more finely divided product. Again, liquid nitrogen is used as needed to maintain solid frozen particles during final grinding. The powder can be easily hydrated using, for example, buffered saline to produce a fluidized tissue graft material of this invention at the desired viscosity.

To prepare another preferred fluidized material, a tela submucosa powder can be sifted through a wire mesh, collected, and subjected to proteolytic digestion to form a substantially homogeneous solution. For example, the powder can be digested with 1 mg/ml of pepsin (Sigma Chemical Co., St. Louis, Mo.) and 0.1 M acetic acid, adjusted to pH 2.5 with HCl, over a 48 hour period at room temperature. After this treatment, the reaction medium can be neutralized with sodium hydroxide to inactivate the peptic activity. The solubilized submucosa can then be concentrated by salt precipitation of the solution and separated for further purification and/or freeze drying to form a protease-solubilized intestinal submucosa in powder shape.

Fluidized tela submucosa compositions of this invention find wide application in tissue replacement, augmentation, and/or repair. The fluidized submucosal compositions can be

used to induce regrowth of natural connective tissue or bone in an area of an existent defect. By injecting an effective amount of a fluidized submucosa composition into the locale of a tissue defect or a wound in need of healing, one can readily take advantage of the biotrophic properties of the tela submucosa. Interestingly, fluidizing SIS by comminution or enzymatic degradation does not result in any appreciable loss of biotrophic activities, as shown in U.S. Pat. No. 5,275,826.

It is also possible to shape large surface area constructs by combining two or more tela submucosa segments of the invention, for instance using techniques as described in U.S. Pat. No. 2,127,903 and/or International Publication No. WO 96/32146, dated Oct. 17, 1996, publishing International Application No. PCT/US96/04271, filed Apr. 5, 1996. Thus, a plurality of tela submucosa strips can be fused to one another, for example by compressing overlapping areas of the strips under dehydrating conditions, to form an overall planar construct having a surface area greater than that of any one planar surface of the individual strips used to shape the construct. Shapes can be made by using sutures, staples, biocompatible adhesives such as collagen binding pastes, or dehydrating overlapping structures then heating the structure as described in U.S. Pat. No. 3,562,820.

As described herein, the invention can take many shapes, such as coiled, helical, spring-like, randomized, branched, sheet-like, tubular, spherical, fragmented, fluidized, comminuted, liquefied, suspended, gel-like, injectable, powdered, ground, sheared, and solid material shape.

The tela submucosa powder can be used alone, or in combination with one or more additional bioactive agents such as physiologically compatible minerals, growth factors, antibiotics, chemotherapeutic agents, antigen, antibodies, enzymes, and hormones. Preferably, the powder-form implant will be compressed into a predetermined, three-dimensional shape, which will be implanted into the bone region and will substantially retain its shape during replacement of the graft with endogenous tissues.

Tela submucosa of the invention can also be used as a cell growth substrate, illustratively in sheet, paste or gel shape in combination with nutrients which support the growth of the subject cells, e.g. eukaryotic cells such as endothelial, fibroblastic, fetal skin, osteosarcoma, and adenocarcinoma cells (see, e.g. International Publication No. WO 96/24661 dated Aug. 15, 1996, publishing International Application No. PCT/US96/01842 filed Feb. 9, 1996). In certain forms, the tela submucosa substrate composition will support the proliferation and/or differentiation of mammalian cells, including human cells.

The inventive tela submucosa can also serve as a collagenous biomaterial in compositions for producing transformed cells, (see, e.g., International Publication No. WO 96/25179, dated Aug. 22, 1996, publishing International Application No. PCT/US96/02136 filed Feb. 16, 1996; and International Publication No. WO 95/22611 dated Aug. 24, 1995, publishing International Application No. PCT/US95/02251 filed Feb. 21, 1995). Such compositions for cell transformation will generally include purified tela submucosa of the present invention, for example in fluidized or paste shape as described in U.S. Pat. No. 5,275,826, in combination with a recombinant vector (e.g. a plasmid) containing a nucleic acid sequence with which in vitro or in vivo target cells are to be genetically transformed. The cells targeted for transformation can include, for example, bone progenitor cells.

In order to promote a further understanding of the present invention and its features and advantages, the following

specific Examples are provided. It will be understood that these specific Examples are illustrative, and not limiting, of the present invention.

EXAMPLE 1

Thirty feet of whole intestine from a mature adult hog is rinsed with water. This material is then treated in a 0.2% by volume peracetic acid in a 5% by volume aqueous ethanol solution for a period of two hours with agitation. The tela submucosa layer is then delaminated in a disinfected casing machine from the whole intestine. The delaminated tela submucosa is rinsed four (4) times with sterile water and tested for impurities or contaminants such as endotoxins, microbial organisms, and pyrogens. The resultant tissue was found to have essentially zero bioburden level. The tela submucosa layer separated easily and consistently from the whole intestine and was found to have minimal tissue debris on its surface.

EXAMPLE 2

A ten foot section of porcine whole intestine is washed with water. After rinsing, this section of tela submucosa intestinal collagen source material is treated for about two and a half hours in 0.2% peracetic acid by volume in a 5% by volume aqueous ethanol solution with agitation. Following the treatment with peracetic acid, the tela submucosa layer is delaminated from the whole intestine. The resultant tela submucosa is then rinsed four (4) times with sterile water. The bioburden was found to be essentially zero.

EXAMPLE 3

A small section of the tela submucosa intestinal collagen material was subcutaneously implanted in a rat. Within 72 hours, significant angiogenesis was observed.

EXAMPLE 4

Two sections of small intestine are processed by differing methods. The first section is rinsed in tap water, disinfected for 2 hours in a 5% by volume aqueous ethanol solution comprising 0.2% by volume peracetic acid, pH approximately 2.6, delaminated to the tela submucosa, rinsed in purified water, divided into two samples and rapidly frozen. The second section is rinsed in tap water, delaminated to the tela submucosa, rinsed in purified water, placed in a 10% neomycin sulfate solution for 20 minutes (as described in U.S. Pat. No. 4,902,508), rinsed in purified water, divided into two samples and rapidly frozen. The four above-prepared samples are tested for bioburden and endotoxin levels. The first two samples each have bioburdens of less than 0.1 CFU/g and endotoxin levels of less than 0.1 EU/g. The second two samples have respective bioburdens of 1.7 CFU/g and 2.7 CFU/g and respective endotoxin levels of 23.9 EU/g and 15.7 EU/g.

EXAMPLE 5

Three sections of small intestine are processed by differing methods. The first is rinsed in tap water, disinfected for 2 hours in a 5% by volume aqueous ethanol solution comprising 0.2% by volume peracetic acid, pH about 2.6, delaminated to the tela submucosa, rinsed in purified water, and rapidly frozen. The second is rinsed in tap water, delaminated to the tela submucosa, rinsed in purified water, disinfected according to the methods of Example 1 in U.S. Pat. No. 5,460,962 (treatment for 40 hours in a 0.1% by volume aqueous solution of peracetic acid, buffered to pH

11

7.2), and rapidly frozen. The third is rinsed in tap water, delaminated to the tela submucosa, rinsed in purified water, disinfected according to the methods of Example 2 in U.S. Pat. No. 5,460,962 (treatment in 0.1% by volume peracetic acid in high salt solution, buffered to pH 7.2), and rapidly frozen. All three samples were tested for endotoxins. The endotoxin levels were <0.14 EU/g for the first sample, >24 EU/g for the second sample, and >28 EU/g for the third sample.

EXAMPLE 6

Two sections of porcine small intestine were infected with 7×10^6 plaque forming units (PFU) of virus. Both were exposed to a 0.18% peracetic acid, 4.8% aqueous ethanol solution at a nine-to-one weight ratio of solution to material. A first sample was immersed in this solution for 5 minutes; the second was immersed for 2 hours. The material processed for 5 minutes exhibited 400 PFU per gram of material. The material processed for 2 hours exhibited zero PFU per gram of material.

EXAMPLE 7

Purified tela submucosa, prepared as described herein, was tested to determine its nucleic acid content. Four samples of material weighing 5 mg each were subjected to DNA/RNA extraction as detailed in the DNA/RNA Isolation Kit by Amersham Lifescience Inc., Arlington Heights, Ill. Nucleic acid quantitation was performed by spectrophotometric determination of solution optical densities at 260 nm and 280 nm. The average nucleic acid content was 1.9 ± 0.2 mg per milligram of material.

Small intestinal submucosa, prepared as described by U.S. Pat. No. 4,902,508, was tested to determine its nucleic acid content. Four samples of material weighing 5 mg each were subjected to DNA/RNA extraction as detailed in the DNA/RNA Isolation Kit by Amersham. Nucleic acid quantitation was performed by spectrophotometric determination of solution optical densities at 260 nm and 280 nm. The average nucleic acid content was 2.4 ± 0.2 mg per milligram of material.

EXAMPLE 8

Sections of tela submucosa prepared according to the methods described herein were sent to an independent testing laboratory (NAMSA, Inc., Northwood, Ohio) for biocompatibility testing as described in the standard ISO 10993. The samples were tested for USP Acute Systemic Toxicity, USP Intracutaneous Toxicity, Cytotoxicity, LAL Endotoxin, material-mediated Pyrogenicity, Direct Contact Hemolysis, and Primary Skin Irritation. The samples passed all tests, indicating that the material is biocompatible.

EXAMPLE 9

Using the procedure set forth in U.S. Pat. No. 5,460,962, two samples were analyzed. The first Kemp sample indicated an endotoxin level greater than 24 endotoxin units per gram and the second Kemp sample indicated an endotoxin level greater than 28 endotoxin units per gram. Thus, when using the procedure set forth in Kemp '962, the endotoxin levels fall outside the biocompatibility levels.

12

EXAMPLE 10

Using the procedures set forth in U.S. Pat. Nos. 4,902,508 and 5,372,821 issued to Badylak, the endotoxin level shown ranges as high as 23.9 endotoxin units per gram per sample. This falls outside the permissible range and thus does not meet the criteria of biocompatibility. The invention, prepared in the above prescribed manner of disinfection first then delamination, was observed to have an endotoxin level of less than 12 endotoxin units per gram, and more particularly, reported an endotoxin level of less than 5 endotoxin units per gram. Thus, the material of the present invention is biocompatible as defined above.

EXAMPLE 11

With reference to FIGS. 2A and 2B, in preparing a lyophilized SIS sponge 10, comminuted SIS (with a mean particle size of approximately $150 \mu\text{m}$) was centrifuged in a Beckman TJ-6 centrifuge with a speed of $1550 \times g$ for 15 minutes. The supernatant was poured off leaving a dough-like consistency of SIS remaining. The material was then poured into various polycarbonate molds and frozen at cold temperatures, preferably at -80°C . for at least 2 hours. The material was then vacuum-dried for 6 hours in a lyophilizing system with the condenser at -70°C . at a vacuum pressure of less than 100 millitorr, preferably 15 millitorr. Optionally, the sponge 10 can then be sterilized using ethylene oxide. The resulting structure was an SIS sponge 10, having a creamyish yellow-white color.

In varying the above procedures, the following attributes were noticed. Varying the "g" force from $0-15000 \times g$, centrifugation at the higher "g" results in an increased sponge 10 density, a decreased sponge absorbency, and increased tensile strength. If the material were sterilized by radiation instead, for example using 10-25 kGy, noted was a decrease in tensile strength. Using comminuted hydrated SIS with a possible fragment size of 25 to $3000 \mu\text{m}$ produced a more condensed, higher density sponge, whereas larger fragments produced a less dense larger pore sized sponge. Adding sugar molecules to the comminuted hydrated SIS resulted in a increased pore size sponge. Furthermore, the addition of cross-linking agents, such as DANECOL, carbodiimide, or glutaraldehyde, caused an increased tensile strength that did not break down as easily upon the addition of water. In addition, moderating the pH of the suspension, from 2-10, preferably 3-5, demonstrated that a higher pH will reduce the tensile strength of the sponge. Since the resulting sponge can take the shape of the mold in which it is placed, modifying the mold shape will modify the sponge shape.

The resulting sponge finds applicability, inter alia, in providing hemostasis in a large wound; fills a large defect; provides a three dimensional structure for cell culture or in vivo growth; provides a plug to stop bleeding; can be an injectable form; can be used for vessel embolization.

EXAMPLE 12

In accordance with the present invention, the lyophilized SIS was compared to other forms of SIS to provide the following data:

	Single layered Lyophilized Sheet	Non De- watered SIS sponge	De- watered SIS Sponge	4-Layered Lyophilized Sheet	4-Layered Vacuum- Pressed Sheet**
Absorbency Level (g water per g of sample)***	5.3	5.6	4.2	3.1	1.0
Density (g/cm ³)	.26	.16	.20	.26	.65
Load at Failure of 1 cm wide strip (gf)	882	161	587	4501	5931
Stress at failure (gf/cm ²)	7737	57	1792	11,169	237,240

*The 4-layered sheet was made in accordance with U.S. Pat. No. 5,711, 969 issued to Patel, Hiles, Whitson, Cheng, Badylak, and Kokini; and assigned to Purdue Research Foundation and Methodist Hospital, all of Indiana, USA. The material used was made in accordance with U.S. Pat. No. 4,902,508, issued to Badylak et al.

***British Pharmacopoeia, 1988, vol. II, App. XX-L, pA226.

Notably, with reference to FIG. 1, the increasing number of lyophilized sheets 15 used indicated a decrease in the absorbency level to approximately 1.01 grams of water per gram of sample. Furthermore, by increasing the amount of water centrifuged out, the density increased.

EXAMPLE 13

With reference to FIG. 1, to prepare lyophilized multilaminar sheets 15, a sheet 20 of hydrated SIS was laid down on a non-stick surface, such as polytetrafluoroethylene (TEFLON(R)) and smoothed to remove any ripples or entrapments between the sheet 20 and the non-stick surface. A second sheet was placed over the first sheet and smoothed out in the same fashion. The process was repeated until the preselected number of sheets 20 were stacked forming the multilaminar sheet structure 15. A thin layer of high purity water was glazed over the top sheet of SIS and the material was frozen at -80° C. for at least 2 hours. The material was then vacuum dried at a vacuum pressure less than 100 millitorr (preferably less than 15 millitorr) in a condenser operating at -70° C. for six hours. The dried sheets were then cut into a preselected shape, sealed in packaging, and terminally sterilized using ethylene oxide.

By modifying the process in the manner described in Example 11, the sheet may have different attributes. For example, it was noted that adjusting the pH of the hydrated SIS to between 2-10, preferably 3-5, demonstrated an increased lamination strength. In addition, a tissue glue may be present between the sheets to increase lamination strength.

EXAMPLE 14

In making the SIS into various forms and lyophilizing the product, it was noted that the porosity index of the SIS changed. A sheet of hydrated SIS was attached to the bottom of a water column exerting a hydrostatic pressure of approximately 35 inches. The amount of water collected via pass through after 30 seconds was measured. The experiment was repeated using a single sheet of lyophilized SIS under the water column. In comparing the hydrated form of a single sheet of SIS with the lyophilized form, the hydrated

form had a mean of 9.59 grams of water collected, with a standard deviation of 5.22. The lyophilized form had a mean of 0.2 grams of water with a standard deviation of 0.13.

With reference to FIGS. 2A and 2B, as with the lyophilized product, the product can be packaged in packages that are, inter alia, gas permeable, hermetically sealed, sterile, sealed, UV protected, double pouched, or some combination thereof. By UV barriered or UV protected, it is meant that the packages or pouches are made of a material that substantially blocks the contents from UV radiation. For example, the package can comprise the material disclosed in U.S. Pat. No. 5,489,022, which issued on Feb. 06, 1996 to Sabin Corp. (Bloomington, Ind.), the disclosure of which is entirely incorporated by reference herein. Preferably, the product is packaged in gas permeable double pouched packages that have a UV barrier to protect the internal product by placing the product in the package and sealing it. Gas permeable packages are desirable as it facilitates terminal sterilization with ethylene oxide. A double pouched package system 25 is desirable in that it permits the user to open the exterior pouch 30 and drop the inner pouch 35 on sterile surfaces. However, the packaging may include a plurality of pouches nested within each other, such that there is a pouch within a pouch within a pouch, etc. with the biomaterial being in the inner or innermost pouch. By sterile, it is meant that the package is sterilized and is of such construction and material as to keep the contents of the package and inner pouches sterile. The package can also contain a buffered solution 50 to keep the product wet. The solution can also contain the pharmacologic agents described above to permit long-term infiltration of the agent into the collagenous biomaterial product. The solution chosen can also include the characteristic that the final package can be terminally sterilized and thus sterilize the solution without deleteriously changing the solution characteristics.

In another embodiment of the invention, the medical device product comprises a submucosa of a warm blooded vertebrate made in the manner described above. The submucosa has an endotoxin level less than 12 endotoxin units per gram of submucosa and the submucosa further having an absorbency level of greater than 1.01 grams of water per gram of submucosa. The collagenous biomaterial further made by the process comprising the steps of sterilizing a source of submucosal tissue from the sources identified above; delaminating the source of submucosal tissue to expose the submucosa; freezing the submucosa in the manners described above; and drying the submucosa under vacuum pressure. Optionally, the collagenous biomaterial may be comminuted by grinding, shearing, or fragmenting the biomaterial in the manner described above. In addition, the collagenous biomaterial can further include packaging the biomaterial in at least one of a gas permeable, sealed, hermetically sealed, sterile, UV protected, and a plurality of pouches.

The collagen biomaterial can be made radiopaque by a variety of conventional procedures, none of which has yet been applied to tela submucosa. In one embodiment of the invention, the collagen material has a shape, namely made into sheets, either in lyophilized or non-lyophilized form. With reference to FIGS. 1, 2A, and 2B, any radiopaque substance 40, including but not limited to, tantalum such as tantalum powder, can be spread along the surface of the tela submucosa, such as on the serosal side. Other radiopaque materials 40 comprise bismuth and barium, including but not limited to, bismuth oxychloride and barium sulphate, as well as other conventional markers. As used herein, the term "disposed" on shall be construed to include disposed on,

15

disposed throughout, disposed in, disposed with, disposed along with, applied on, applied with, applied through, applied in, applied in conjunction with, and the like. With particular reference to tela submucosa, the differential porosity of the material can enable more radiopaque material 40 to be disposed on the tela submucosa.

In one particular embodiment, radiopaque marker tantalum powder was disposed on a sheet of tela submucosa by rubbing it onto the serosal side of the tela submucosa. The tela submucosa was then made into various shapes, such as, but not limited to, having the shape of a brush-like, braided, branched, helical, spherical, cubic, cylindrical, tubular, injectable, randomized, layered, and sheet-like shapes. For example, an injectable shape of the invention can be readily made by comminuting the invention into small fibrils, fragments, or the like, then suspending them in solution, such as, but not limited to, a biocompatible gelatin suspension. Due to the viscosity of the gelatin suspension, the invention, when injected into the lumen of an aneurysm, will stay in the lumen and provide the therapeutic benefit to the aneurysm.

The invention and collagenous biomaterial can be made in layers. In this manner, the collagenous material can increase its structural integrity, strength, and applicability. In one embodiment of the invention, a dual layer of collagenous biomaterial can be used in sheets and either the radiomarker or pharmacologic agent, or both can be disposed in between the layers.

In similar fashion, the pharmacologic agent 45 can be disposed on the collagenous material. As used herein, the pharmacologic agent 45 includes, but is not limited to, growth factors, proteins, proteoglycans, glycosaminoglycans, physiological compatible minerals, antibiotics, chemotherapeutic agents, enzymes, drugs, and hormones. The agent can be disposed on the serosal or mucosal sides of the submucosa, or can be disposed on the same or opposite sides of the collagenous material. Consideration of placement can be important depending on the desired shape of the final device. For example, where the shape is tubular, it can be desirable to place the pharmacologic agent 45 on the abluminal surface since that surface will be in contact with the surrounding tissue. On the other hand, if systemic release of the agent 45 is contemplated, then the agent can be placed on the luminal side to permit the blood to contact the agent and carry it away. Utilizing the braid shape, each individual strip can be treated with different agents.

It will be appreciated that variations of the above-described processing procedures are intended to be within the scope of this invention. For example, the source tissue for the collagenous biomaterial, e.g., stomach, whole intestine, cow uterus and the like, can be partially delaminated, treated with a disinfecting or sterilizing agent followed by complete delamination of the tela submucosa. Illustratively, attached mesentery layers, and/or serosa layers of whole intestine can be advantageously removed prior to treatment with the disinfecting agent, followed by delami-

16

nation of remaining attached tissues from the tela submucosa. These steps can or can not be followed by additional disinfection steps, e.g., enzymatic purification and/or nucleic acid removal. Alternatively, the tela submucosa source can be minimally treated with a disinfecting or other such agent, the tela submucosa delaminated from the tunica muscularis and tunica mucosa, followed by a complete disinfection treatment to attain the desired contaminant level(s). All such variations and modifications are contemplated to be a part of the process described herein and to be within the scope of the invention.

Many alterations and modifications can be made by those of ordinary skill in the art without departing from the spirit and scope of the invention. The illustrated embodiments have been shown only for purposes of clarity and examples, and should not be taken as limiting the invention as defined by the appended claims, which include all equivalents, whether now, or later devised.

What is claimed is:

1. A collagenous biomaterial medical device, comprising: a laminate having a plurality of layers of lyophilized submucosa;

said submucosa having an absorbency level of greater than 1.01 grams of water per gram of submucosa and a density between about 0.10 and about 0.40 grams per cubic centimeter.

2. The collagenous biomaterial medical device of claim 1, wherein the submucosa has an absorbency level of greater than 2.0 grams of water per gram of submucosa.

3. The collagenous biomaterial medical device of claim 2, wherein the submucosa is a member selected from the group consisting of small intestinal submucosa, stomach submucosa and urinary bladder submucosa.

4. The collagenous biomaterial medical device of claim 3, wherein the submucosa is small intestinal submucosa.

5. A collagenous biomaterial medical device, said collagenous biomaterial medical device comprising a laminate structure including submucosa having an absorbency level of greater than 1.01 grams of water per gram and a density between about 0.10 and about 0.40 grams per cubic centimeter, said laminate structure prepared by a process comprising:

providing a plurality of submucosa layers in contact with one another;

lyophilizing the plurality of submucosa layers in contact with one another.

6. The collagenous biomaterial medical device of claim 5, wherein the submucosa has an absorbency level of greater than about 2 grams of water per gram.

7. The collagenous biomaterial medical device of claim 6, wherein the submucosa is a member selected from the group consisting of small intestinal submucosa, stomach submucosa and urinary bladder submucosa.

8. The collagenous biomaterial medical device of claim 7, wherein the submucosa is small intestinal submucosa.

* * * * *